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Natasha Ali
Aga Khan University

Bushra Moiz
Aga Khan University

Yasmin Rehman
Aga Khan University

Mohammad Salman
Aga Khan University

Shahid A Sami
Aga Khan University

Original Article

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Natasha Ali,[1] Bushra Moiz,[2] Yasmin Rehman,[3] Mohammad Salman,[4] Shahid A Sami[5]

Department of Pathology & Microbiology,[1-4] Department of Surgery,[5] The Aga Khan University Hospital, Karachi, Pakistan.

Abstract

Objective: To determine the frequency of Heparin Induced Thrombocytopenia (HIT) in patients undergoing elective cardiac bypass surgeries and to observe the platelets evolution post surgically in these patients.

Method: A prospective study was designed and 100 unselected consecutive patients undergoing elective cardiac bypass surgery were enrolled and evaluated . The clinical and laboratory details were noted before and after surgery. Their platelet counts were observed from day 0 (prior to surgery) to day 5 post operatively. Particle gel immunoassay was used to demonstrate the presence of antibodies against heparin-platelet factor 4 complexes.

Results: There were 86 males and 14 females with median age of 59 and 53.5 years respectively. Marked platelet count variation was detected during post operative period in all patients (n=100) ranging from 23 ± SD186.73 to 389 ± 84.12 X 109/L. However, 20 patients showed >50% drop on day 5 and seven of these also showed seroconversion. The clinical scoring for HIT was found to have a good negative predictive value. Thromboembolic complications were not observed in any of our patients.

Conclusions: HIT is prevalent to a significant extent in patients after cardiac surgery in our clinical setting though it mostly remains undetected and is an under-diagnosed entity. (JPMA 59:345; 2009)

Introduction

Heparin induced thrombocytopenia (HIT) Type II is a life threatening pro-thrombotic complication of heparin treatment resulting from immunoglobulin G (IgG) antibodies that bind to platelet Fc RIIA receptors inducing platelet activation with subsequent thrombocytopenia, thrombin generation and endothelial cells injury.[1] In sharp contrast, HIT type I is a benign reversible disorder with mild thrombocytopenia where platelet counts often recover in spite of continued heparin treatment.[2] Typically, onset of immune mediated thrombocytopenia is observed after 5 days of heparin exposure and may either resolve within a fortnight of drug discontinuation but such platelet recovery may be delayed for months in some patients.[2]

HIT type II has been reported in both surgical and medical settings. HIT occurs in up to 5% of orthopaedic surgery patients, 3% of cardiac surgery patients, more than 1% of vascular surgery patients and 1% of medical patients receiving therapeutic doses of unfractionated heparin.[3] The reasons for these variations are unknown but could be related to different heparin preparations, patient population dependent factors or even different laboratory techniques used in detection of HIT associated antibodies. Patients undergoing cardiac surgery on cardiopulmonary bypass (CPB) have inherent risk of strong platelet activation due to release of large amounts of platelet factor 4 (PF4) in their plasma.[4] Additionally, exposure to large doses of unfractionated heparin makes these patients unduly susceptible to HIT.[5] This perhaps is the reason of development of HIT associated antibodies in nearly 50% of them.[6] In spite of this seroconversion, clinical manifestations of HIT are observed in only 3% of CPB patients.[6]

Heparin-induced thrombocytopenia should be viewed as a clinicopathologic syndrome, that is, the diagnosis should be made only if clinical abnormalities are seen and HIT antibodies are detected.[7] The clinical criteria include exposure to unfractionated or low molecular weight heparin, thrombocytopenia or a 50% decrease in baseline platelet count, exclusion of other causes of thrombocytopenia (e.g. medications, infections, disseminated intravascular coagulation), presence or absence of thrombosis, atypical features in a minority of cases (e.g. skin lesions, systemic reactions to heparin), and recovery of thrombocytopenia after cessation of heparin exposure.[8] In vitro demonstration of heparin-platelet factor 4 antibodies (H-PF4) can be done through various functional and immunological assays.[9] Although the former tests (serotonin release and platelet aggregation assay and flow cytometry) are more specific and gold standard for the diagnosis of HIT but they at the same time are also technically demanding. In contrast, commercially available antigen essays (e.g. enzyme linked immonosorbent assay tests [ELISA] or particle gel immunoassay) are rapid and user-friendly with ability to detect antibodies (IgG, IgM and

IgA classes) that bind to PF4/heparin or PF4/polyvinylsulfate complexes bound to a solid phase.

This study was designed to determine the frequency of HIT in patients undergoing elective cardiac surgery on cardiopulmonary bypass and to correlate HIT with the trend in platelet count during immediate post operative period.

Patients and Methods

This was a prospective study conducted between September 2006 and August 2007 at Aga Khan University Hospital. We enrolled 100 unselected consecutive patients undergoing elective on-pump cardiac surgery in the study after approval from institutional ethical review committee and informed consent of the patients. Clinical details for each enrolled patient were carefully recorded with special emphasis on any previous exposure to unfractionated (UFH) or low molecular weight heparin (LMWH). Patients having thrombocytopenia (platelet count <100X109/L) or thrombocytosis (platelet count >450 x 109/L) at the start of study were not registered. Similarly patients with any underlying malignancy, or those receiving chemotherapy or radiotherapy were also excluded.

Dosage and type of heparin received during and after surgery was also noted.

Blood samples were collected from each patient in gel tube (BD, Becton Dickinson and Company, New Jersey, USA) for detection of H-PF4 antibodies and in EDTA container (5.0 ml in each) for platelet counts. Briefly, platelet count was determined in each patient pre-operatively and on 3rd and 5th post operative day. The platelet counts were repeated if considered necessary at any time during their 7 days hospital stay or during their subsequent follow-up. Their sera were tested for H-PF4 antibodies prior to and during 5-7 days after surgery.

A particle gel immunoassay ID-Heparin/PF4 antibody test (ID-HPF4) (DiaMed, Cressier sur Morat, Switzerland) was utilized for the detection of antibodies to heparin-PF4 complexes. Briefly, 10µL of test serum was added to 50µL of pre-prepared suspension of red high density polystyrene beads coated with heparin-PF4 complexes into the reaction chamber containing a buffered sephacryl gel matrix and incubated for 5 minutes at room temperature followed by centrifugation in ID centrifuge. H-PF4 antibodies were detected by agglutinated beads either on top or being dispersed through the gel matrix. A positive test was repeated to ensure consistency of results. In addition, complete blood count was performed for each patient by Coulter Gen-S (Coulter Electronics, Fullerton, CA, USA) and Leishman stained peripheral film was also examined by experienced technologists and haematologists to rule out pseudo thrombocytopenia. Prothrombin time (PT), activated partial thromboplastin time (APTT) and plasma glucose levels were retrieved from our hospital based computerized data system.

Anticoagulation for cardiopulmonary bypass was achieved with bolus intravenous infusion of 400 units/kg of sodium UFH (porcine) just before cardiac intervention. Additional heparin was administered with continuous IV infusion with the aim to keep activated clotting time (measured through Medtronic ACT II) above 500 seconds during surgery by monitoring the test at half hourly interval. LMWH was not received by any patient. On completion of surgery, reversal of heparinization was accomplished through intravenous infusion of protamine sulphate. The mean duration of CPB for patients was 1.45 hours. The patients were further anticoagulated after surgery only if INR was <2.0 and patient had undergone an additional valve replacement. This was achieved with continuous heparin infusion.

The accepted criteria for the diagnosis of HIT type II[8] is different from that described for CPB.[10] Accordingly, we considered a normal pattern of PC if the counts returned to normal within 5-6 days of surgery. A diagnosis of HIT was considered by either a decrease in PC that follows a previous correction of thrombocytopenia occurring during CPB (pattern a) or a persistently low PC in the days following surgery (pattern b). Laboratory evidence for the presence of circulating HIT antibodies was also determined. Clinical records were observed by two subjects individually to determine the patterns of platelet drop post surgically. Clinical probability of heparin induced thrombocytopenia was calculated objectively through Greinacher scoring system.[11] Additionally, Warkentin and Heddle score system[12] was also utilized to determine the same. In the first system three categories are defined: unlikely (score 0-3), probable (score 4, 5) and highly probable or definite (score 6 or more) while in later the scores are: 6-8 (high), 4-5 (intermediate) and 0-3 (low).

SPSS statistical software Version 16.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Two tailed probability test (Mann-Whitney) and paired t test were utilized where considered necessary to compare groups. Level of significance was 0.05. Comparisons between HIT and no- HIT subgroups were made using stepwise logistic regression analysis.

Results

We evaluated 100 unselected consecutive patients undergoing CPB including 14 females (median age 59 years, range 45 to 73) and 86 males (median age 53.5, range 52 to 56) for heparin induced thrombocytopenia. Of these, 4 patients underwent an additional valve replacement or repair procedure. Co-morbids like hypertension, diabetes and ischaemic heart disease was present in 43, 47 and 21 patients respectively. Of

100 patients that were enrolled, only 19 did not have significant family history for associated medical problems. Physical examination was unremarkable in all patients.

Prior to surgery, laboratory investigations showed; platelet count 224.79±80.79 x 10^9/L (range 100 - 411 10^9/L), random blood sugar levels 139.48±61.73mg/dl (range 70-339mg/dl) while prothrombin (PT) and activated partial thrombin times (APTT) were 12.31±2.15 (range 10.30-31.80) and 26.83±2.98 (range 20.0-40.00) seconds respectively. Gender wise demographics and clinical details of patients did not show any statistically significant variable with respect to sex (data not shown).

Platelet drop:

Marked platelet count variation was detected during post operative period in all patients (n=100) ranging from 23 ± 186.73 to 389 ± 84.12 X 10^9/L. Sixteen patients showed more than 50% drop in their platelet count from their baseline on day 3 while another four patients did so in next two days. Hence there were 20 patients on day 5 who showed >50% decline in their platelet count. Their platelet counts (X 10^9/L) on day 0 were 100-411 (269±93.299) while on day 5 were 23-182 (92.80±46.385). Seven of these patients were positive for H-PF4 antibodies. Percentage drop of platelet counts post operatively is summarized in Table 1 and Figure. This shows

Table 1: Number of CPB patients with platelet trend during immediate post op stay (n=100).

Platelet drop	n	Day 0	Day 5	p-value
>50 % decrease*	20	100-411(269±93)	23-182(92±46)	0.000
30-50% decrease	11	170-411(280±80)	89-269(170±51)	0.000
Normal	53	150-389(225±58)	150-389(241±62)	0.000
Low platelets	16	103-148(123±15)	65-250(127±50)	0.848

*The group includes one patient from low pre-operative platelet count.

that there was statistically significant difference in platelet drop in 31 patients showing either >50% drop (n=20) or 30-50%drop from their baseline (n=11). There were 53 patients who had normal platelets to begin with and they showed a statistically significant rise in their platelet counts on day 5. There were 16 patients who had low platelet count (<150X10^9/L) preoperatively and showed a rising trend postoperatively though not statistically significant. The patients with platelet drop were also evaluated for other co-existing causes of thrombocytopenia such as septicaemia, drugs and massive blood transfusion through logistic regression analysis (data not shown). None of these were identified as a possible etiology in any of the patients.

Frequency of H-PF4 antibodies:

All 100 patients were tested for H-PF4 antibodies before and after surgery. Plasma samples from all patients

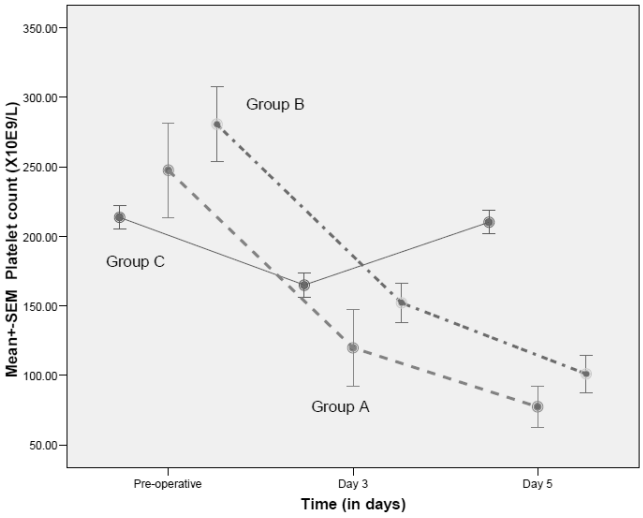

Figure: Trend in the platelet counts in CPB patients showing 50% drop on 5th post op day in patients with (group A; n=7) and without HIT antibodies (group B; n=13). Group C (n=64) showed either 30-50% platelet dip or normal recovery. Data of 16 patients who had low platelet count at day 0 is not shown.

(100/100) were negative for these antibodies prior to surgery. However, on day 5-7 post surgically, H-PF4 antibodies were positive in seven patients (7%). All seven patients had >50% decrease in their platelet counts on day 5 from their initial baseline.

Clinical HIT and seroconversion:

We characterized our patients into two groups as per criteria used by Pouplard et al in 2005.[10] Accordingly, 64 patients were considered to be normal as all of them showed recovery of their platelet count by day 5; 20 patients showed a drop in platelet count during the surgical procedure and failed to show recovery to normal values in the postoperative period. Of these patients, 7 showed seroconversion as anti H-PF4 antibodies were detected in their sera on day 5-7. There were 16 patients with preoperative platelet count between 100-150X10^9/l who were not evaluated due to their lower counts though seven of these patients did show a significant drop in their platelet counts on day 5.

The clinical probability scorings for HIT as measured through two systems: Warkentin and Heddle and Greinacher[11,12] are summarized in Table 2. It was observed that patients who were diagnosed HIT on the basis of platelet evolution (n=20) had statistically significant platelet drop in post surgical period (p 0.000) and had probability scoring of "high" (n=7) and "intermediate" (n=10) as per Warkentin protocol and "likely" (n=7) and "unlikely" (n=13) according to system devised by Greinacher. The first system was found to better correlate with clinical HIT based on platelet evolution than the second method. Moreover, both scoring systems were found to have a good negative predictive value as all patients

Table 2: Probability of Clinical HIT as determined through Pouplard platelet evolution, Warkentin and Greinacher scoring system. n=100.

Clinical Diagnosis	Platelet Count		Sig.(2-tailed)	Warkentin	Greinacher
	Day 0	Day 5			
HIT with seroconversion (n=7)	262.43±78.14	78.14±38.749	0.000	Low 0 Intermediate 4 High 3	Unlikely 4 Likely 3 Definite 0
HIT without seroconversion (n=13)	262.50±86.70	103.33±33.950	0.000	Low 3 Intermediate 6 High 4	Unlikely 9 Likely 4 Definite 0
Normal platelet evolution (n=64)	240.78±69.336	232.95±61.632	0.443	Low 64 Intermediate 0 High 0	Unlikely 64 Likely 0 Definite 0
Low platelet counts on Day 0(n=16)	122.47±16.394	116.29±57.343	0.669	*	*

*Data not analyzed for scoring.

who had normal platelet recovery after CPB were identified to have "low" score by Warkentin and were predicted as "unlikely" by Greinacher. No statistically significant difference was observed in patients with HIT (n=20) and HIT (n=64) had no association with age and gender.

Thromboembolic complications:

No thromboembolic phenomenon was observed in any patient in the immediate post operative period. Also, none of the patients developed skin necrosis or systemic reaction to heparin.

Discussion

The number of cardiac by pass surgeries performed in our institute is estimated to be 750 annually. A number of patients were assessed to be thrombocytopenic after cardiac surgery but etiology of low platelets was never formally evaluated in our clinical setting. This study was designed to evaluate the frequency of typical onset HIT during on pump elective CPB after exposure to UFH.

It has been observed that a significant number of CPB patients demonstrate transient thrombocytopenia post surgically due to haemodilution and/or platelet activation from contact of blood with extracorporeal circuit.[13] Immune mediated HIT can further complicate the scenario by inducing thrombocytopenia with paradoxical life threatening thrombosis. Three different clinical presentations have been described: rapid, typical, and delayed onsets HIT where platelets drop begin respectively at day 1, 5 -14, and 9-40 after cardiac surgery.[14]

A very variable incidence of typical onset HIT ranging from 29% to 61% has been documented in CPB patients.[15] We diagnosed HIT clinically in 19 patients according to platelet evolution as described previously.[10] However, only 7 of these had serological evidence of HIT. There may be several reasons for the low frequency of HIT as observed by us: absence of prior exposure to heparin within 100 days before CPB (except 2 patients), administration of relatively small dosage of heparin during surgery and evaluation of H-PF4 antibodies during day 5-7 only. In addition, methodology for detection of HIT associated antibodies in this study was also dissimilar to that used in other studies.

We utilized particle gel immunoassay for anti H-PF4 antibodies for detection of HIT associated antibodies. The reason for this choice may be convenience, decrease turn around time and technical simplicity. Although functional assay like 14C-Serotonin release assay (SRA) is considered to be gold standard and is specific for the detection of HIT antibodies,[9] the requisite technical sophistication for this test restricted us from such work up. Commercial PF4/polyanion enzyme immunoassay are sensitive for detection of HIT antibodies though they are less specific as may be detectable in 50% of CPB patients after one week of surgery. Moreover results of ID-HPF4 had been observed to be comparable to functional assays like SRA and heparin induced platelet aggregation assay (HIPA) tests[16] with an impressive sensitivity of 94%.[17] However, it is recommended that gel test should be used in combination with a functional assay as other antigens involved in HIT such as interleukin 8 or neutrophil-activating peptide 2 may not be detected by it.[18]

Monitoring of platelet counts is an important critical requirement of post cardiac surgical care to enable the early diagnosis of HIT. In our experience, 64 of 100 patients demonstrated lowering of their platelet count from initial baseline on day 3-5 and patients who developed anti H-PF4 antibodies had platelet count ranging from 23-124 (mean 77.43 ± 39.85). We found that a drop of >50% platelet on post day 5 irrespective of initial baseline count was closely associated with the development of H-PF4 antibodies. Although median platelet count reported in HIT associated with cardiac surgery was as low as 55-60 x 10^9/L,[19] however 10% of such patients never show a platelet nadir of less than 150 x 10^9/L which may be because HIT complicates a postoperative course that would otherwise be characterized by thrombocytosis.[10] Pouplard et al in 2005[10] studied 305 patients with cardiac intervention and noted an incidence of 53.4% HIT with platelet drop of 50%

during day 8-11.

Patients with HIT (n=20) were classified as having "probable" or "intermediate" and "high risk" for HIT by Greinacher and, Warkentin and Heddle Scoring systems respectively. However, 13 /20 patients were "unlikely" to have HIT according to Greinacher.[11] We found this system more difficult to use and correctly apply to our patients as platelet recovery within 10 days as required by the scoring system could not be evaluated. Both systems correlated very well in normal patients (without HIT) where scoring was either "unlikely" or "low". Hence these systems were found to have excellent negative predictive value.

Generally, heparin induced thrombocytopenia is strongly associated with venous and arterial thrombosis. About 40%-50% patients with thrombocytopenia alone (isolated HIT) will eventually develop a thrombotic event.[20] The overall thrombotic risk is very variable and has been related to the severity of platelet decline ranging from 50% in mildly thrombocytopenic patients (platelet nadir >100 x 10^9/L) to 90% in patients with severe thrombocytopenia (platelet nadir, <30 x 10^9/L).[21] In sharp contrast, thrombotic events are not that frequently reported in cardiac surgery. Visentin et al[21] in 1996 found an incidence of 61% HIT in CPB but did not observed thromboembolic complication in any of his patients. Similarly, Pouplard et al[10] in 2005 reported aortic thrombosis in only one of 305 cardiac surgery patients. Occasional cases of thrombosis in CPB have been reported by a few.[22-24] In our experience, five of seven patients who developed H-PF4 antibodies had mean platelet count of 99.2 ± 17.58 while remaining two had 23X10^9/L. In spite of these low platelet counts, none of them had any thrombotic episode. This may be associated to genetic variation related to our population. A study on 389 patients with HIT, FcγIIa genotype was identified as the risk factor for developing HIT associated antibodies and thrombotic complications.[25]

Limitations of Study:

Earlier discharge of patient after cardiac surgery makes prompt recognition of HIT difficult. Consequently, we could not evaluate for typical onset HIT beginning after that time.

HPF4 ELISA detecting IgG only and C14 serotonin release assays have high sensitivity and specificity respectively. However, these tests were not utilized during the study as they are technically demanding and are not available in our set up or in any reference laboratory of our country.

Conclusions

This study demonstrates that heparin induced thrombocytopenia is a relatively common adverse effect of unfractionated heparin therapy during cardiopulmonary bypass surgery in our setting. Careful follow-up of platelet counts may identify patients who will eventually develop HIT antibodies. Larger prospective studies are needed to evaluate true dimensions of the disease utilizing sophisticated tests. There is also a need to study the various polymorphisms linked to genetic variation with the possible development of HIT associated antibodies.

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